



# Multicomponent crystals of fenofibric acid-*L*-proline with enhanced dissolution rate and antihyperlipidemic activity

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**ABSTRACT:** The objectives of this study were to prepare and characterize a novel fenofibric acid-*L* prolin multicomponent crystals and to evaluate the improvement in dissolution rate and antihyperlipidemic activity of fenofibric acid when prepared in the multicomponent crystal formation. The solid-state characterization of the novel multicomponent crystal was performed by powder X-ray diffraction (XRD), differential scanning calorimetry (DSC), scanning electron microscopy (SEM) and Fourier transform-infrared (FT-IR) spectroscopy. The multicomponent crystals of fenofibric acid (FA) and *L*-prolin (PR) was prepared by solvent drop grinding method. Dissolution rate profiles were evaluated in phosphate buffer pH 6.8. To investigate the in vivo antihyperlipidemic activity, 16 male Swiss-Webster rat were injected with cholesterol-inductor solution 1%, following oral administration of intact fenofibric acid 9,45 mg/kg, physical mixture, and multicomponent crystals fenofibric acid- *L*-proline (equivalent to 9,45 mg/ kg fenofibric acid). The plasma level of cholesterol was determine with photometer. All data were analyzed with two-way ANOVA followed by Tukey HSD test (95% confidence interval). The results of the antihyperlipidemia test showed that the cholesterol levels of animals treated with multicomponen crystals FA-PR lower than the cholesterol levels of animals treated with fenofibric acid and physical mixture (significance <0,05). The Solubility and dissolution rate of multicomponent crystals FA-PR were also improved significantly compared to intact fenofibric acid.

**KEYWORDS:** Fenofibric acid ; *L*-Proline ; Multicomponen crystals ; Solubility ; Dissolution rate.

## 1. INTRODUCTION

Fenofibric acid (FA) is an antihyperlipidemic mainly used in treatment lower cholesterol levels. Due to poor solubility in water, fenofibric acid was classified as a BCS class II drug according to Biopharmaceutical Classification System (low solubility and high permeability). Currently, more than 40% of new drugs on the market have low water solubility. Therefore, it is important to make efforts to increase drug solubility which leads to an increase in oral bioavailability [1].

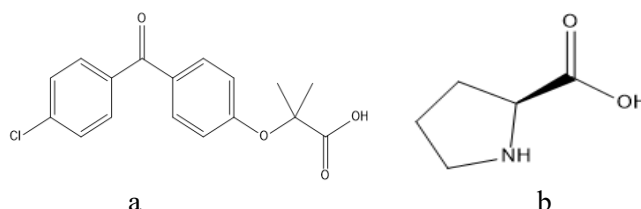
Several methods have been developed to increase the solubility of fenofibric acid, including the addition of an alkalizing agent  $MgCO_3$ , made a mixture with  $MgCO_3$  in a 2:1 mol ratio [2], the formation of a ternary solid dispersion of fenofibric acid with hyaluronic acid and polyethylene glycol [3], Formation of fenofibric acid salt using choline base, diethanolamine, trometamine, calcium, ethanolamine, and piperazine [4], surface solid dispersion using croscarmellose sodium [5], and formation of fenofibric acid self nanoemulsion to increase the dissolution rate [6]. Solubility enhancement by multicomponent crystalline technique offers many advantages, among which is that the solid material is a stable crystalline phase and will affect many physicochemical properties of the solid material

Crystal engineering techniques with multicomponent crystal formation can improve the physicochemical properties of drug compounds such as solubility, dissolution rate, stability and compressibility by modifying the crystal structure without change its pharmacological activity [7]. Multicomponent crystals can be designed by selecting suitable excipients (coformers) that can interact with drug molecules by noncovalent bonds to form new crystalline phases [8]. The formation of multicomponent crystals of fenofibric acid has not been reported.

In this investigation, we prepared multicomponent crystals fenofibric acid (FA) (Figure 1A) and coformer *L*-proline (PR) (Figure 1B) by solvent drop grinding method. The coformer *L*-proline is used

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because theoretically the carboxylic group on L-proline has the potential to form a homosynthon with the carboxylic group on fenofibric acid and solvent drop grinding is a widely used method in crystal engineering techniques. Solid characterisation of multicomponent crystals formed characterized by powder X-Ray diffraction, differential scanning calorimetry, Fourier Transform infra red spectroscopy, and scanning electron microscopy analysis (SEM), solubility and dissolution behavior. This study also looked at the effect of fenofibric acid multicomponent crystal formation on increasing pharmacological activity.

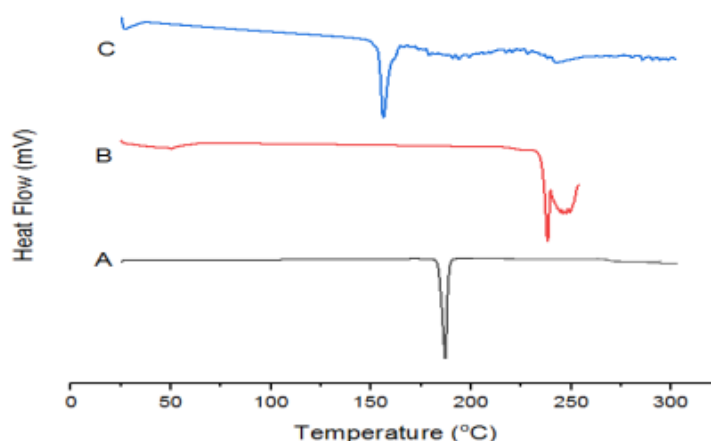


**Figure 1.** Molecular structure of (a) fenofibric acid (b) L-Proline

## 2. RESULTS AND DISCUSSION

Thermal analysis is used to evaluate the thermodynamic changes that occur when a material is given heat energy, including melting, recrystallization, desolvation and transformation into the solid phase can be observed by endothermic or exothermic peak on DSC thermogram [9]. The DSC curves and the data of the melting temperature-enthalpy of fusion ( $\Delta H$ ) are shown in Figure 2 and Table 1, respectively.

The DSC curve of FA showed a single endothermic peak at 185,36°C ( $\Delta H = 192.8$  J/g), while the PR showed a single endothermic peak at 234.80°C ( $\Delta H = 19,19$  J/g). This endothermic peak is the melting point of FA and PR. Indication of new formation the crystal phase is the emergence different melting point by initial component [10]. FA-PR multicomponent crystals has a lower melting point than the melting point of both the individual components. The DSC curve of FA-PR multicomponent crystals has a melting endothermic peak at 156.11°C ( $\Delta H = 67.17$  J/g) while the FA and PR respectively endotherm peak is 185,36 °C ( $\Delta H = 192,80$  J/g), 234.80 °C ( $\Delta H = 19,195$  J/g), markedly different from the melting endothermic peak and enthalpy of FA and PR. These results indicate that fenofibric acid and L-proline transforms into multicomponent crystals to form a new crystalline phase. In addition, the DSC curve with a single endothermic peak indicates the absence of components are not bound because the mixture is complete transforms into the crystalline phase of multicomponent crystal [11].

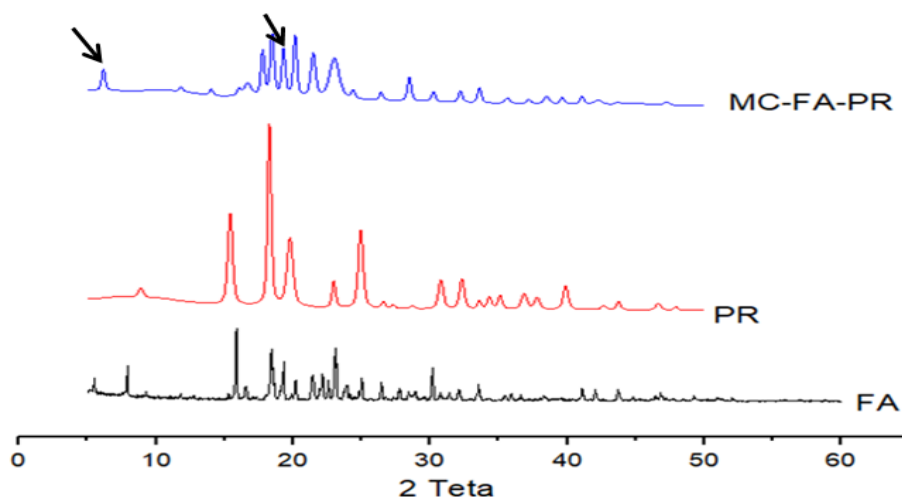


**Figure 2.** DSC curve A) fenofibric acid B) L-proline C) FA-PR multicomponent crystals

**Table 1.** Melting temperature and enthalpy of fusion of fenofibric acid, L-proline and FA-PR multicomponent crystals

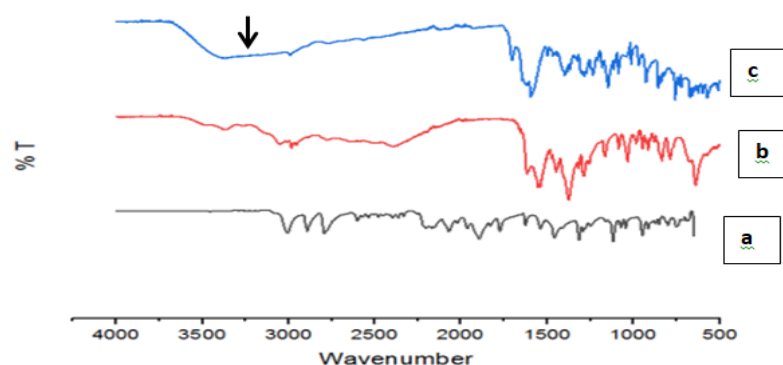
Samples	Melting temperature (°C)	Enthalpy of fusion ( $\Delta H$ , J/g)
Fenofibric acid	185.36	192.80
L-proline	234.80	19.19
FA-PR Multicomponent crystals	156.11	67.17

PXRD is an important method in characterization of crystalline properties of solid samples. The formation of new crystalline phases can be confirmed based on different diffractogram patterns [12]. The characteristic diffraction of multicomponent crystals is characterized by the appearance of new diffraction peaks and the absence of some diffraction peaks of individual components [13]. The overlay of powder X-ray diffractograms of FA, PR and FA-PR multicomponent crystals is shown in Figure 3. The diffractogram of FA has specific peaks of at  $2\theta = 18,6$ ;  $23,1$  and  $23,3$ , while PR has  $2\theta$  specific peaks at  $15,3$ ;  $18,3$ ;  $19,8$ ; and  $25$ . The diffractogram of FA-PR multicomponent crystals exhibits new peaks at position  $2\theta 5,9^\circ$  and  $17,7^\circ$ , which were absent in both the fenofibric acid and the L-proline. The powder X-ray diffractogram of showed diffraction peaks different from those of the constituents. The diffraction peaks of indicated the difference of its crystal lattice compared to the individual components. This indicates that fenofibric acid L-proline have formed multicomponent crystals with new solid crystalline [13].



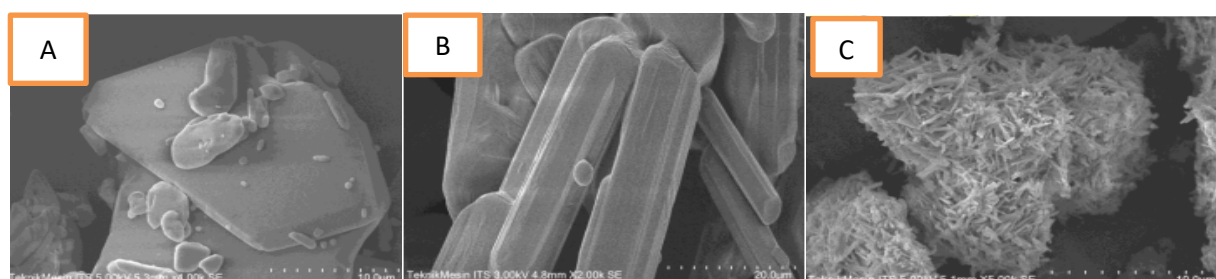
**Figure 3.** Powder X-Ray diffractogram of pure fenofibric acid (FA), L-proline (PR) and multicomponent crystals fenofibric acid -L-proline (MC-FA-PR)

Fourier transformation infra red (FT-IR) is a technique commonly used to characterize intermolecular interactions in multicomponent crystals (Kumar, 2019). The obtained FTIR spectra of FA, PR and FA-PR multicomponent crystals are shown in Figure 4. FTIR spectrum of FA has absorption peaks at  $2990\text{ cm}^{-1}$  and  $1700\text{ cm}^{-1}$  (corresponding to C-H stretching and  $\text{-C=O}$  ketone stretching). The FTIR spectra of L-proline has specific peaks at  $3373\text{ cm}^{-1}$  and  $2982\text{ cm}^{-1}$  (corresponding to O-H stretching and C-H stretching). The spectra of multicomponent crystals FA- showed a shift of the absorption peaks compared to the spectra of individual components. The shift of the absorption peaks of FA-PR multicomponent crystals at a wave number of  $3382\text{ cm}^{-1}$  indicates the presence of an OH functional group which indicates that between the form of fenofibric acid and the coformer L-proline can form hydrogen bonds which indicates that multicomponent crystals have formed [14].



**Figure 4.** FTIR spectra of (a) fenofibric acid (b) L-proline (c) FA-PR multicomponent crystals

The SEM images of FA, PR and the FA-PR multicomponent crystals are presented in Figure 5. The test results with SEM show that the crystals habit of FA is like a flat plate, PR like a cylindrical plate with a regular shape, while the FA-PR changes the habit of crystals formed in the form of agglomerates with solid chunks inside like needles. The morphological changes of individual components in the FA-PR multicomponent crystals indicated the formation of a multicomponent crystal between FA and PR.



**Figure 5.** SEM image of (A) FA (B) L-proline (C) FA-PR multicomponent crystals

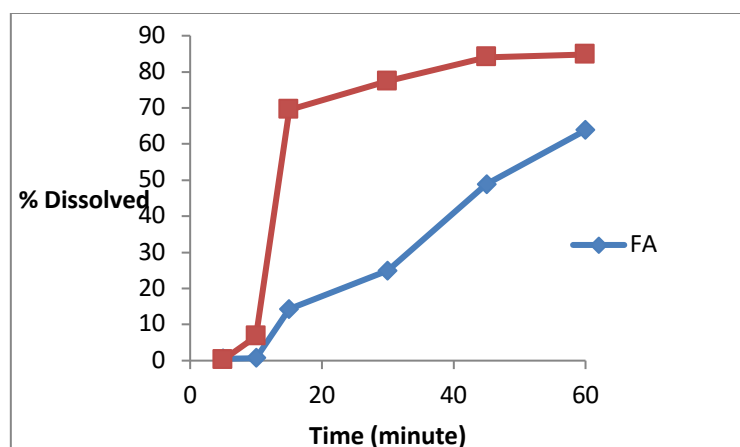
The solubility assesment of fenofibric acid and FA-PR multicomponent crystal in distilled water was 66.82  $\mu\text{g/mL}$  and 244.26  $\mu\text{g/mL}$  respectively. FA-PR multicomponent crystal showed a significant increase in solubility ( $p < 0.05$ ) of about 3.6 times higher compared to the solubility of fenofibric acid. The increase of solubility of the multicomponent crystal is often associated with a decrease of its melting point. A multicomponent crystal with a lower melting point tends to have higher solubility than the initial ingredient [15]. The DSC experiment showed that the FA-PR multicomponent crystal has a melting point at 156.11°C, which is lower by 29.25°C than fenofibric acid. So it can be expected that the increased solubility of the FA-PR multicomponent crystal may be caused by the decrease of the melting point.

**Table 2.** Solubility of fenofibric acid (FA) and multicomponent crystal FA-PR

Compound	Solubility ( $\mu\text{g/mL}$ )	$\pm\text{SEM}$	Increased solubility
FA	66.82	0.07	-
FA-PR	244.26	0.55	3.6 times

The dissolution behaviour of fenofibric acid and FA-PR multicomponent crystal in phosphate buffer pH 6.8 was shown in Figure 6. The multicomponent crystal showed higher dissolution rate compared to fenofibric acid with a cumulative amount released 84.79% at 60 minutes. The dissolution profile in distilled water indicated that the amount of fenofibric acid released from the FA-PR multicomponent crystal was higher than the pure fenofibric acid (only about 63.8%). Decreased crystallinity in multicomponent crystals contributes to less intermolecular interactions in the crystal lattice. The lower the molecular interactions in

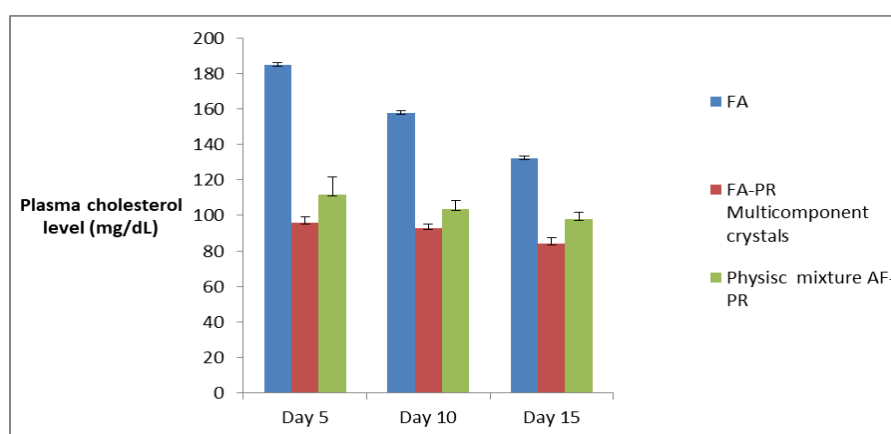
the crystal lattice, the lower the energy of the crystal, so the amount of enthalpy decreases as the melting point decreases. Similar results of increased solubility were also shown in other studies using L-proline as a coformer using other active pharmaceutical ingredients [16].



**Figure 6.** Dissolution rate profile of FA and multicomponent crystals FA-PR

Evaluation of antihyperlipidemic activity in this study using rat and CHOD/PAP method (*cholesterol oxydase- peroxydase aminoantipyrin*). This method is one of the most commonly used techniques for evaluate antihyperlipidemic activity. Rat cholesterol was induced by injecting inductor solution orally. Inductor solution consisting of cholesterol, propylthiouracil, and peanut oil. This solution is known to induced rat cholesterol [17]. Plasma cholesterol levels were determined after 5, 10 and 15 days of drug administration. The plasma cholesterol levels of experimental animals were compared before being given the drug and after being given the drug. The in vivo evaluation of antihyperlipidemic activity in this study showed that multicomponent crystals FA-PR was effective in lowering cholesterol compared to the intact FA and its physic mixture.

The process of absorption of BCS II drugs such as FA in the gastrointestinal tract limited by its low solubility and slow dissolution rate. The low solubility of this drug may result in incomplete absorption even though the membrane permeability is good [18]. The formation of multicomponent crystals FA-PR can overcome this limitation. This study shows an increase in solubility which can simultaneously enhance the antihyperlipidemic effect in experimental animals.



**Figure 7.** Comparative average plasma cholesterol level between groups  $p < 0.05$  as compare with control group (analyzed with Duncan's MRT following two-way ANOVA with 95% confidence interval  $n = 15$ )

### 3. CONCLUSION

The results of the study conclude that the preparation of multicomponent crystals fenofibric acid with L-proline significantly enhances the solubility and dissolution of this poorly soluble drug. The mixture also improves the antihyperlipidemia effect, as compared with intact fenofibric acid and its physical mixture.

### 4. MATERIALS AND METHODS

#### 4.1 Material

Fenofibric acid was purchased from BOC Sciences (New York, USA), L-proline was purchased from (Tokyo, Chemical Industri), acetonitrile and ethanol HPLC grade purchased from Merck (Germany). CHOD-PAP reagent, water used was double-distilled. All other solvents used in this study research were of analytical grade.

#### 4.2 Method

##### 4.2.1 Preparation Physic Mixture and Multicomponent Crystals by Solvent Drop Grinding Method

Multicomponent crystals FA-PR was prepared by a solvent drop grinding method of 1:1 molar ratio. Three drops of ethanol was added and the mixture was ground manually by using a pestle until the solvent evaporated; this process was repeated, and the resultant sample was referred to as the liquid-assisted grinding (LAG) sample.

##### 4.2.2 Solid-State Characterization

###### a. Differential Scanning Calorimetry (DSC) Analysis

Thermal analysis of the samples was performed by using differential scanning calorimetry (Shimadzu DSC-60 Plus, Japan). A sample eutectic mixture of 5-7 mg is placed in a closed aluminum pan. The DSC apparatus is programmed over a temperature range of 30 to 250 °C with a heating rate of 10 °C per minute.

###### b. Powder X-Ray Diffraction Analysis

Powder X-ray diffraction analysis of the samples were carried out at room temperature using a PANalytical PW 30/40 X-ray diffractometer (the Netherlands). The measurement conditions are as follows: metal Cu, K $\alpha$  filter, voltage 40 kV, current 40 mA, an analysis was performed in the range 2 theta 10 - 40 °. The diffractograms were processed using Origin Lab software.

###### c. Fourier Transform-Infrared Spectroscopy Analysis

Intermolecular reactions were analyzed using FT-IR spectroscopy (Thermo scientific, USA). The sample was mixed with KBr with a ratio of 1:100 and this mixture was compressed to form pellets. Absorption of samples is recorded at wave numbers between 4000 - 600 cm<sup>-1</sup>. Analyzes was performed for intact FA, PR and the multicomponent crystals of FA-PR.

###### d. Scanning Electron Microscopy Analysis

Microscopic analysis of eutectic mixture using an SEM apparatus (HITACHI type Flexsem 1000, Japan). The sample is placed in the sample holder and all samples were sprayed with a thin film of gold-palladium. Measurement conditions; 10 kV voltage and 12 mA.

##### 4.2.3 Solubility Test

The saturated solubility in CO<sub>2</sub> free distilled water was determined at room temperature using an orbital shaker. Excess amounts of the samples were added to 100 mL of the media, and then filtered through a membrane filter after 24 h of equilibration. The concentration of fenofibric acid was determined by the HPLC technique carried out in triplicates. The analysis was performed by using HPLC Shimadzu (Japan) equipped with DAD UV-Vis detector filled with 5  $\mu$ m material. A mixture of acetonitrile and water (70:30)



was used as the mobile phase. Fenofibric acid was detected by UV spectrophotometer at wavelength 287 nm. The retention time (tR) of fenofibric acid was 6.187 min.

#### 4.2.4 *In-vitro* Dissolution Rate Profile

Dissolution rate profiles were determined by using USP type 1 dissolution apparatus (SR8-Plus Hanson Research, USA) at 100 rpm and  $37 \pm 0.5$  °C. Dissolution medium was phosphate buffer pH 6.8 900 mL. The aliquots were withdrawn after 5, 10, 15, 30, 45 and 60 minutes. Each solution was filtered with PTFE 0.45 µm. The analysis was performed by using HPLC SHIMADZU (Japan) equipped with DAD UV-Vis detector. The HPLC system consists of columns pursuit XRS C18 4.6 × 125 mm. A mixture of acetonitrile and water adjust pH 3 (70:30) was used as the mobile phase.

#### 4.2.5 *In Vivo* Evaluation of Antihyperlipidemia Activity

##### a. Animal Preparation

A number of 15 male wistar rat aged 3 months and weighed 250-300 g were use for this study. The animals were kept under standard environmental conditions at room temperature for 10 days of acclimatization. The experimental protocol was approved by the Ethics Committee of Faculty of Medicine, Andalas University No. 61/UN.16.2/KEP-FK/2020.

##### b. Antihyperlipidemia Activity Evaluation

The evaluation of antihyperlipidemia activity was conducted by the cholesterol oxidase method. Previously, all experimental animals were induced with an inductor solution for 7 days to increase serum cholesterol levels. The animal were divided into control and treatment group. The treatment groups were subdivided into 3 groups receiving intact FA, physical mixture of FA-PR, and multicomponent crystals of FA-PR meanwhile the control animal group was treated with 1% Na CMC. All doses were administered by oral gavage at a dose equivalent to 9.45 mg/kg body weight.

##### c. Determine Serum Cholesterol Levels

Serum cholesterol levels were determined on day 5, 10 and 15 after being given treatment. Blood is drawn through a vein in the eye. Blood sample is put into clot/gel activator tube. Centrifuge at 3000 rpm for 15 minutes. Take the serum with a micropipette and transfer it to a microtube and add CHOD-PAP reagent. Determine the cholesterol level with photometer.

#### 4.2.6 Statistical Analysis

Data from the experiment were presented as mean ± SEM. Statistical analysis was performed by using two-way ANOVA followed by Tukey HSD Test. The significance level was taken at 95% of confidence interval. All statistical analyses were carried out using SPSS version 1

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